Stress-Enhanced Fear Learning: A Model of Comorbid PTSD and Substance Use

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Introduction

Post-traumatic stress disorder (PTSD) and substance use disorder (SUD) are highly comorbid, with estimates as high as 50-65% concomitance in veterans. Our laboratory has shown that comorbidity can be modeled in rats utilizing an acute stressor, repeated unsignaled footshock, with shocked rats showing an increased sensitivity to drug-related cues, higher baseline anxiety, and increased fear expression following mild stress in a new context[1].

Numerous experiments have shown that Pavlovian conditioning plays a significant role in mediating drug relapse[2]. To test for the ability of an acute stress to influence drug-seeking, we used a well-characterized model of substance use, drug self-administration. Rats infused methamphetamine intravenously for 2 weeks following acute footshock stress. Each infusion was concurrent with illumination of a cue light above the active lever. A second lever was present but without programmed consequences. After stable response was acquired, the lever press to light/meth contingency was severed (extinction). The ability of the drug-paired cue light to initiate drug seeking was then assessed.

Mice were used in a conditioned place preference (CPP) study[3]. CPP induces a Pavlovian association between a context and the unconditioned stimulus of a drug. Preference for a drug-paired context versus a neutral context is used to assess how reinforcing that drug is for the animal, with pleasurable drugs leading to CPP and unpleasant drugs leading to avoid of the paired floor.

Materials and Methods

Subjects

- 78 male Long-Evans rats were utilized for rat experiments, with those self-administering meth undergoing surgery to place a catheter in their right jugular vein for IV infusions. 24 male C57Bl/6J mice were used for CPP experiments. Rats and mice were approximately 10-12 weeks old prior to start.
- Single-housed

Apparatus & Procedure

Fear

- Fear conditioning occurred in a novel context, in a separate room from any appetitive conditioning.
- Shocks were 1.0 mA for 1 second in duration. SEFL procedure was 15 unsignaled footshocks over 90 minutes, pseudorandom variable inter-stimulus interval with an average of 6 minutes between shocks.

Operant Self-Administration

- At the start of each session, two levers would extend. One would be randomly designated the active lever prior to the start of the experiment, and would remain the active lever for that animal for all sessions.
- Pressing of the active lever would lead to intravenous methamphetamine infusion, as well as the lighting of a cue light directly above the active lever. Infusion and cue-light would continue for 5 seconds.

Conditioned Place Preference

- Over 8 days, mice received 4 pairings of cocaine and 4 pairings of saline with a floor type randomly chosen at the beginning of the experiment. Mice were restricted to the particular floor of that day. On test days mice had access to both floor types. Conditioning and test sessions were 15 minutes in duration.

Statistical Analysis

- All statistical analyses were performed via repeated measures two-way ANOVA (Group x Session), with Bonferroni-corrected post-hoc tests, except test of cued reinstatement, dexamethasone suppression test, and elevated plus maze (one-way ANOVA).

Shock induces long-term enhancement of fear

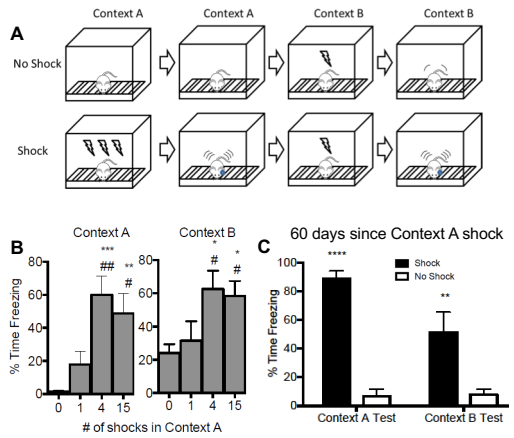


Figure 1. Acute stress in one context enhances fear in a second context. A) Schematic of the SEFL procedure. Animals receive either 15 or 0 footshocks in Context A, and are tested for freezing behavior 24 hours later. One day after that, all groups receive a single shock in a new context, Context B. Shock in Context A enhances fear learning in Context B. **B)** Animals shocked once in Context B freeze in Context B, but those that received 4 or 15 footshocks in Context A freeze significantly more. **C)** Shock and effect of shock are long-lasting, remaining at least 60 days since original shock in Context A. # = p<0.05 compared to 1 shocks in Context A, ## = p<0.01 compared to 1 shocks. *, **, *** = p<0.05, p<0.01, p<0.01 compared to 0 shocks, respectively.

Shock causes an anxious phenotype

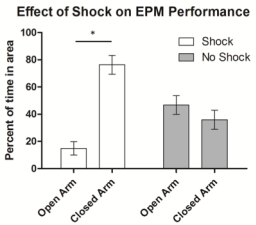


Figure 2. Acute stress induces anxiety as assessed by elevated plus maze. Test of elevated plus maze over 5 minutes, one week after shock in Context A. Rats that had received 15 footshocks showed significantly more anxiety-like behavior than rats that had not been shocked.

Shock doesn't cause HPA axis dysregulation

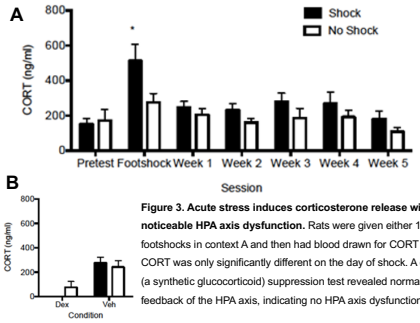


Figure 3. Acute stress induces corticosterone release without noticeable HPA axis dysfunction. Rats were given either 15 or 0 footshocks in context A and then had blood drawn for CORT analysis. CORT was only significantly different on the day of shock. A dexamethasone (a synthetic glucocorticoid) suppression test revealed normal negative feedback of the HPA axis, indicating no HPA axis dysfunction.

Acute stress enhances cues associated with drug-seeking

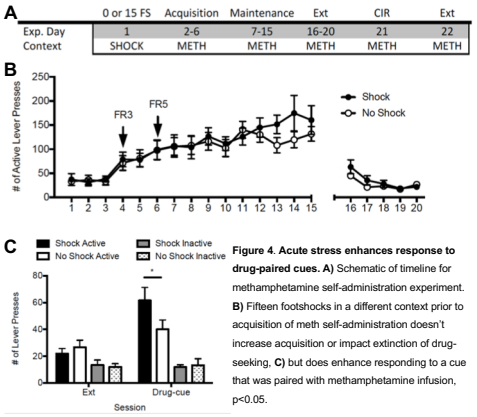


Figure 4. Acute stress enhances response to drug-paired cues. A) Schematic of timeline for methamphetamine self-administration experiment. **B)** Fifteen footshocks in a different context prior to acquisition of meth self-administration doesn't increase acquisition or impact extinction of drug-seeking, **C)** but does enhance responding to a cue that was paired with methamphetamine infusion, p<0.05.

Stress effects are cross-species

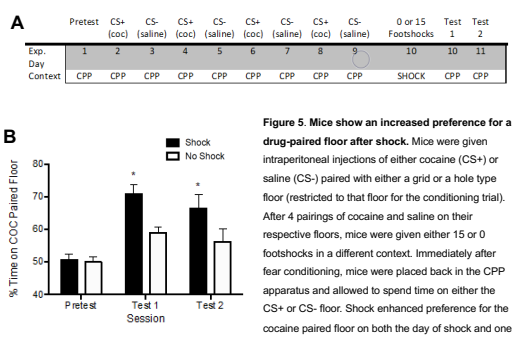
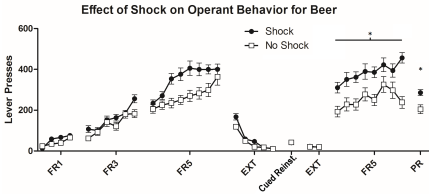


Figure 5. Mice show an increased preference for a drug-paired floor after shock. Mice were given intraperitoneal injections of either cocaine (CS+) or saline (CS-) paired with either a grid or a hole type floor (restricted to that floor for the conditioning trial). After 4 pairings of cocaine and saline on their respective floors, mice were given either 15 or 0 footshocks in a different context. Immediately after fear conditioning, mice were placed back in the CPP apparatus and allowed to spend time on either the CS+ or CS- floor. Shock enhanced preference for the cocaine paired floor on both the day of shock and one day later (p<0.05).

Conclusions/Future Directions

Acute stress in the form of unsignaled footshock leads to long-term changes in behavior in animals, including an increased sensitivity to future fearful events, increases in anxiety, and enhancements in the ability of drug-paired cues to initiate drug-seeking. While there is an increase in stress response (as assessed by corticosterone levels), this increase is only in response to the acute stress and not due to general increased HPA axis activity or dysfunction. It remains unclear what drives these long-term changes after a traumatic event, whether these differing behavioral outcomes have a similar neurological locus, and what predisposes some to PTSD while others show resilience.

Future work will look at the acquisition of these symptoms (predisposition to drug abuse, anxiety, and hyper-responsiveness to perceived threats) and how trauma induces such behavior. A strong candidate region is the basolateral amygdala (BLA), which is required for the acquisition and expression of all of these behaviors. Future work will involve selective manipulation of this region during acute stress to attempt to disentangle what role the BLA plays in each altered behavior. Additionally, future work will look at alcohol in particular (the most widely abused drug for PTSD patients[4]) as an abused substance, and whether acute stress shows similar effects with alcohol self-administration. Preliminary data suggests that shock instead affects the amount of alcohol preferred and the willingness to work for it (below), rather than a response to alcohol-related cues, but more research is required.



References/Funding

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